

11/10/00



JC839 U.S. PTO

11-13-00

A

JC839 U.S. PTO

09/709237



11/10/00

Please type a plus sign (+) inside this box → ☐Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.**UTILITY
PATENT APPLICATION
TRANSMITTAL**

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	30195-pa
First Inventor	Coelho, P., et al.
Title	Apparatus and Method of Preparation of Stable, Long...
Express Mail Label No.	EL217260257US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ☒ Fee Transmittal Form (e.g., PTO/SB/17)
(Submit an original and a duplicate for fee processing)
2. ☒ Applicant claims small entity status.
See 37 CFR 1.27.
3. ☒ Specification [Total Pages 20]
(preferred arrangement set forth below)
 - Descriptive title of the invention
 - Cross Reference to Related Applications
 - Statement Regarding Fed sponsored R & D
 - Reference to sequence listing, a table, or a computer program listing appendix
 - Background of the Invention
 - Brief Summary of the Invention
 - Brief Description of the Drawings (if filed)
 - Detailed Description
 - Claim(s)
 - Abstract of the Disclosure
4. ☒ Drawing(s) (35 U.S.C. 113) [Total Sheets 12]
5. Oath or Declaration [Total Pages 4]
 - a. ☐ Newly executed (original or copy)
 - b. ☒ Copy from a prior application (37 CFR 1.63 (d))
(for continuation/divisional with Box 17 completed)
 - i. ☐ **DELETION OF INVENTOR(S)**
Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
6. ☐ Application Data Sheet. See 37 CFR 1.76

ADDRESS TO:Assistant Commissioner for Patents
Box Patent Application
Washington, DC 20231

7. ☐ CD-ROM or CD-R in duplicate, large table or Computer Program (Appendix)
8. Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)
 - a. ☐ Computer Readable Form (CRF)
 - b. Specification Sequence Listing on:
 - i. ☐ CD-ROM or CD-R (2 copies); or
 - ii. ☐ paper
 - c. ☐ Statements verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

9. ☐ Assignment Papers (cover sheet & document(s))
10. ☐ 37 CFR 3.73(b) Statement of Power of Attorney (when there is an assignee)
11. ☐ English Translation Document (if applicable)
12. ☒ Information Disclosure Statement (IDS)/PTO-1449 ☒ Copies of IDS Citations
13. ☒ Preliminary Amendment
14. ☒ Return Receipt Postcard (MPEP 503) (Should be specifically itemized)
15. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed)
16. ☒ Other: Letter to the Draftsman

17. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in a preliminary amendment, or in an Application Data Sheet under 37 CFR 1.76:

☐ Continuation ☒ Divisional ☐ Continuation-in-part (CIP)
Prior application information: Examiner Robinson, H.of prior application No.: 09, 129,988
Group / Art Unit: 1653

For CONTINUATION OR DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 5b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

18. CORRESPONDENCE ADDRESS☐ Customer Number or Bar Code Label

(Insert Customer No. or Attach bar code label here)

or ☒ Correspondence address below

Name	Bernhard Kreten				
Address	77 Cadillac Drive, Suite 245				
City	Sacramento	State	California	Zip Code	95825
Country	United States	Telephone	(916) 921-6181	Fax	921-9213

Name (Print/Type)	Bernhard Kreten	Registration No. (Attorney/Agent)	27,037
Signature			Date 11/9/2000

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS SEND TO Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231

CERTIFICATE OF MAILING UNDER 37 C.F.R. 1.10

Applicant: Philip Henry Coelho, Phil Kingsley, Jim Brausch, James H. Godsey and Gail Rock

For: Apparatus and Method of Preparation of Stable, Long Term Thrombin from Plasma and Thrombin Formed Thereby

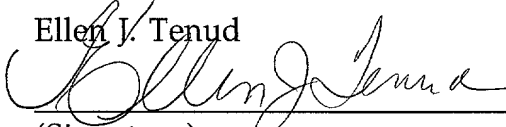
Paper:

1. A Patent Application (Utility) (comprised of pages 1 through 20) (copy from a prior application);
2. A Utility Patent Application Transmittal;
3. A Fee Transmittal (original and one copy);
4. A Declaration for Patent Application (copy from a prior application);
5. A Verified Statement Claiming Small Entity Status (Independent Inventor) (copy from a prior application);
6. Twelve (12) sheets of drawing figures (comprised of figures 1 through 12) (copy from a prior application);
7. A Preliminary Amendment;
8. A Letter to the Draftsman;
9. Twelve (12) sheets of formal drawing figures (comprised of figures 1 through 12);
10. A Form PTO-1449 (including prior art copies); and
11. A check in the amount of \$435.00, \$355.00 of which reflects the government filing fee for utility patent, and \$80.00 of which is to cover the government fee for two (2) independent claims in excess of three.

I hereby certify that the above identified correspondence, which is attached, is being deposited with the **United States Postal Service, Express Mail, Post Office to Addressee, mailing label #EL217260257US**, in an envelope addressed to:

Assistant Commissioner for Patents
Box Patent Application
Washington, D.C. 20231

on November 10, 2000.

Ellen J. Tenud

(Signature)
November 10, 2000
(Date of Signature)

FEE TRANSMITTAL for FY 2000

Patent fees are subject to annual revision.

TOTAL AMOUNT OF PAYMENT (\$) 435.00

Complete if Known

Application Number
Filing Date
First Named Inventor Coelho, P., et al.
Examiner Name
Group Art Unit
Attorney Docket No. 30195-pa

METHOD OF PAYMENT (check one)

1. ☒ The Commissioner is hereby authorized to charge indicated fees and credit any overpayments to:
- Deposit Account Number 11-1734
Deposit Account Name Bernhard Kreten
- ☒ Charge Any Additional Fee Required Under 37 CFR 1.16 and 1.17
☒ Applicant claims small entity status. See 37 CFR 1.27
2. ☒ Payment Enclosed:
☒ Check ☐ Credit card ☐ Money Order ☐ Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description	Fee Paid
101 690	201 345	Utility filing fee	355.
106 310	206 155	Design filing fee	
107 480	207 240	Plant filing fee	
108 690	208 345	Reissue filing fee	
114 150	214 75	Provisional filing fee	

SUBTOTAL (1) (\$) 355.

2. EXTRA CLAIM FEES

Total Claims 12
Independent Claims 5
Multiple Dependent

Extra Claims Fee from below Fee Paid
-20** = 0 X =
-3** = 2 X 40. = 80.

**or number previously paid, if greater; For Reissues, see below

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description
103 18	203 9	Claims in excess of 20
102 78	202 39	Independent claims in excess of 3
104 260	204 130	Multiple dependent claim, if not paid
109 78	209 39	** Reissue independent claims over original patent
110 18	210 9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) (\$) 80.

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description	Fee Paid
105 130	205 65	Surcharge - late filing fee or oath	
127 50	227 25	Surcharge - late provisional filing fee or cover sheet	
139 130	139 130	Non-English specification	
147 2,520	147 2,520	For filing a request for <i>ex parte</i> reexamination	
112 920*	112 920*	Requesting publication of SIR prior to Examiner action	
113 1,840*	113 1,840*	Requesting publication of SIR after Examiner action	
115 110	215 55	Extension for reply within first month	
116 380	216 190	Extension for reply within second month	
117 870	217 435	Extension for reply within third month	
118 1,360	218 680	Extension for reply within fourth month	
128 1,850	228 925	Extension for reply within fifth month	
119 300	219 150	Notice of Appeal	
120 300	220 150	Filing a brief in support of an appeal	
121 260	221 130	Request for oral hearing	
138 1,510	138 1,510	Petition to institute a public use proceeding	
140 110	240 55	Petition to revive - unavoidable	
141 1,210	241 605	Petition to revive - unintentional	
142 1,210	242 605	Utility issue fee (or reissue)	
143 430	243 215	Design issue fee	
144 580	244 290	Plant issue fee	
122 130	122 130	Petitions to the Commissioner	
123 50	123 50	Petitions related to provisional applications	
126 240	126 240	Submission of Information Disclosure Stmt	
581 40	581 40	Recording each patent assignment per property (times number of properties)	
146 690	246 345	Filing a submission after final rejection (37 CFR § 1.129(a))	
149 690	249 345	For each additional invention to be examined (37 CFR § 1.129(b))	
179 690	279 345	Request for Continued Examination (RCE)	
169 900	169 900	Request for expedited examination of a design application	

Other fee (specify)

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) (\$) 0.

SUBMITTED BY

Name (Print/Type) Bernhard Kreten

Registration No. 27,037
(Attorney/Agent)

Complete (if applicable)

Telephone (916) 921-6181

Signature

Date 11/9/2000

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

Applicant: Philip Henry Coelho, Phil Kingsley, Jim Brausch, James H. Godsey, Gail Rock
Serial or Patent No.: 09/129,988 Attorney's Docket No.: 28182-pa
Filed: August 5, 1998
For: Apparatus and Method of Preparation of Stable, Long Term Thrombin from Plasma and Thrombin Formed Thereby

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY
STATUS (37 CFR 1.9(f) and 1.27(b)) - INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees under section 41 (a) and (b) of Title 35, United States Code, to the Patent and Trademark described in:

 the specification filed herewith.
XX application serial no.: 09/129,988, filed August 5, 1998.
 patent no. , issued .

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign grant, convey or license any rights in the invention is listed below:

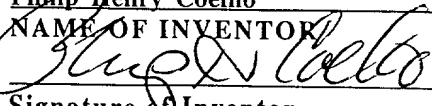
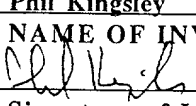
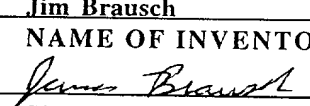
 no such person, concern, or organization.
XX person, concerns or organizations listed below*

*NOTE: Separate verified statements are required for each named person, concern or organization having rights to the invention averring to their status as small entities (37 CFR 1.27)

FULL NAME ThermoGenesis Corp.
ADDRESS 3146 Gold Camp Drive, Rancho Cordova, California 95670
☐ individual ☒ small business concern ☐ nonprofit organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon or any patent to which this verified statement is directed.

<u>Philip Henry Coelho</u>	<u>Phil Kingsley</u>	<u>Jim Brausch</u>
NAME OF INVENTOR	NAME OF INVENTOR	NAME OF INVENTOR
		
Signature of Inventor	Signature of Inventor	Signature of Inventor
<u>10/6/98</u>	<u>10/6/98</u>	<u>10/6/98</u>
Date	Date	Date

Gail Rock

NAME OF INVENTOR

NAME OF INVENTOR

Signature of Inventor

Signature of Inventor

Signature of Inventor

Signature of Inventor

10-6-98

Date _____

Date _____

Date _____

1. General Information	
Item	Value
1.1. Name of the Project	...
1.2. Date of the Report	...
1.3. Author's Name	...
1.4. Institution	...
1.5. Address	...
1.6. Phone Number	...
1.7. E-mail Address	...
1.8. Website	...
1.9. Fax Number	...
1.10. Other Contact Information	...
1.11. Summary of the Project	...
1.12. Objectives of the Project	...
1.13. Scope of the Project	...
1.14. Methodology of the Project	...
1.15. Results of the Project	...
1.16. Conclusions of the Project	...
1.17. Recommendations of the Project	...
1.18. Acknowledgments	...
1.19. References	...
1.20. Appendix	...
1.21. Glossary	...
1.22. Index	...
1.23. Bibliography	...
1.24. List of Figures	...
1.25. List of Tables	...
1.26. List of Equations	...
1.27. List of Symbols	...
1.28. List of Abbreviations	...
1.29. List of Acronyms	...
1.30. List of Initials	...
1.31. List of References	...
1.32. List of Figures	...
1.33. List of Tables	...
1.34. List of Equations	...
1.35. List of Symbols	...
1.36. List of Abbreviations	...
1.37. List of Acronyms	...
1.38. List of Initials	...
1.39. List of References	...
1.40. List of Figures	...
1.41. List of Tables	...
1.42. List of Equations	...
1.43. List of Symbols	...
1.44. List of Abbreviations	...
1.45. List of Acronyms	...
1.46. List of Initials	...
1.47. List of References	...
1.48. List of Figures	...
1.49. List of Tables	...
1.50. List of Equations	...
1.51. List of Symbols	...
1.52. List of Abbreviations	...
1.53. List of Acronyms	...
1.54. List of Initials	...
1.55. List of References	...
1.56. List of Figures	...
1.57. List of Tables	...
1.58. List of Equations	...
1.59. List of Symbols	...
1.60. List of Abbreviations	...
1.61. List of Acronyms	...
1.62. List of Initials	...
1.63. List of References	...
1.64. List of Figures	...
1.65. List of Tables	...
1.66. List of Equations	...
1.67. List of Symbols	...
1.68. List of Abbreviations	...
1.69. List of Acronyms	...
1.70. List of Initials	...
1.71. List of References	...
1.72. List of Figures	...
1.73. List of Tables	...
1.74. List of Equations	...
1.75. List of Symbols	...
1.76. List of Abbreviations	...
1.77. List of Acronyms	...
1.78. List of Initials	...
1.79. List of References	...
1.80. List of Figures	...
1.81. List of Tables	...
1.82. List of Equations	...
1.83. List of Symbols	...
1.84. List of Abbreviations	...
1.85. List of Acronyms	...
1.86. List of Initials	...
1.87. List of References	...
1.88. List of Figures	...
1.89. List of Tables	...
1.90. List of Equations	...
1.91. List of Symbols	...
1.92. List of Abbreviations	...
1.93. List of Acronyms	...
1.94. List of Initials	...
1.95. List of References	...
1.96. List of Figures	...
1.97. List of Tables	...
1.98. List of Equations	...
1.99. List of Symbols	...
1.100. List of Abbreviations	...
1.101. List of Acronyms	...
1.102. List of Initials	...
1.103. List of References	...
1.104. List of Figures	...
1.105. List of Tables	...
1.106. List of Equations	...
1.107. List of Symbols	...
1.108. List of Abbreviations	...
1.109. List of Acronyms	...
1.110. List of Initials	...
1.111. List of References	...
1.112. List of Figures	...
1.113. List of Tables	...
1.114. List of Equations	...
1.115. List of Symbols	...
1.116. List of Abbreviations	...
1.117. List of Acronyms	...
1.118. List of Initials	...
1.119. List of References	...
1.120. List of Figures	...
1.121. List of Tables	...
1.122. List of Equations	...
1.123. List of Symbols	...
1.124. List of Abbreviations	...
1.125. List of Acronyms	...
1.126. List of Initials	...
1.127. List of References	...
1.128. List of Figures	...
1.129. List of Tables	...
1.130. List of Equations	...
1.131. List of Symbols	...
1.132. List of Abbreviations	...
1.133. List of Acronyms	...
1.134. List of Initials	...
1.135. List of References	...
1.136. List of Figures	...
1.137. List of Tables	...
1.138. List of Equations	...
1.139. List of Symbols	...
1.140. List of Abbreviations	...
1.141. List of Acronyms	...
1.142. List of Initials	...
1.143. List of References	...
1.144. List of Figures	...
1.145. List of Tables	...

Gail Rock

NAME OF INVENTOR

Signature of Inventor

Date _____

UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Coelho, P., et al.	}
		}
FOR:	Apparatus and Method of	}
	Preparation of Stable, Long	}
	Term Thrombin From Plasma	}
	and Thrombin Formed Thereby	}
		}

To: Commissioner of Patents and Trademarks
Washington, DC 20231

PRELIMINARY AMENDMENT

Sir:

Before a first Office Action on the merits, kindly enter the following amendments:

IN THE SPECIFICATION

Page 2, line 1, kindly insert the paragraph -- This application is a division of application serial number 09/129,988, filed August 5, 1998, status: pending. -- after "Field of the Invention".

Page 11, line 15, kindly change "CaCL₂" to -- CaCl₂ --.

Page 11, line 17, kindly change "CaCL₂" to -- CaCl₂ --.

Page 11, line 24, kindly change "CaCL₂" to -- CaCl₂ --.

Page 12, line 7, kindly change "CaCL₂" to -- CaCl₂ --.

Page 13, line 10, kindly change "fluids" to --fluid's--.

IN THE CLAIMS

Kindly cancel claim 13 without prejudice or disclaimer as to its content.

///

Kindly Modify the Claims as Follows:

Claim 4, line 1, kindly change "includes" to -- occurs by --.

Claim 4, line 2, kindly change "CaCL₂" to -- CaCl₂ --.

Claim 6, line 1, kindly insert -- altering the clotting time, yielding -- after "wherein".

Claim 10, line 1, kindly insert -- thrombin -- after "A".

Claim 10, line 1, kindly delete -- for extracting thrombin from plasma -- after "composition".

Claim 12, line 3, kindly insert -- only -- after "adding".

Kindly Amend the Claims as Follows:

Claim 1 (amended) - A method for generating [autologous] thrombin from a sole donor or a patient, the steps including:

obtaining a blood product from the patient;

sequestering plasma from the product;

maintaining the plasma undiluted and unprocessed;

enriching the prothrombin in a [plasma] fraction of the undiluted, unprocessed plasma;

converting the prothrombin to thrombin, and

filtering particulate from the thrombin.

Claim 3 (amended) - The method of claim 2 [including] wherein the enriching step occurs by adding ethanol to enrich the prothrombin in a plasma fraction.

Claim 8 - (amended) A method for producing autologous thrombin which is stable for more than 15 minutes, the steps including:

sequestering pro[-]thrombin from plasma using ethanol and converting the pro[-]thrombin to thrombin by adding calcium ions.

Claim 9 - (amended) Autologous thrombin which provides fast clotting
of less than five seconds which is stable for more than 15 minutes.

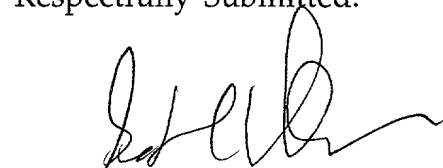
REMARKS

This Preliminary Amendment is provided before receipt of any substantive Office Action on the merits in this case and is provided to rectify various minor typographical inexactitudes and to present amended claims for the Examiner's kind consideration. No new matter has been presented.

In view of the foregoing, it is respectfully requested that the Examiner enter these amendments into this case.

Dated: November 9, 2000

Respectfully Submitted:



BERNHARD KRETEN
Applicant's Attorney
Telephone (916) 921-6181
Registration No.: 27,037

Figure 1 is a schematic representation of the experimental design. It is divided into two main sections: 'Pre-Test' and 'Main Experiment'. The 'Pre-Test' section includes 'Pre-Test 1' (N=10) and 'Pre-Test 2' (N=10). The 'Main Experiment' section includes 'Main Experiment 1' (N=20) and 'Main Experiment 2' (N=20). Each section shows a sequence of steps: 'Stimulus presentation', 'Response', 'Feedback', and 'Inter-trial interval'. The 'Pre-Test' section also includes 'Practice trials' and 'Feedback'. The 'Main Experiment' section includes 'Practice trials' and 'Feedback'. The 'Main Experiment 1' section also includes 'Stimulus presentation' and 'Response'.

—

BACKGROUND OF THE INVENTION

Formulation of a fibrin sealant mimics the last step of the coagulation cascade wherein the enzyme thrombin cleaves fibrinogen which is then cross-linked into a semi-rigid or flexible fibrin clot. This fibrin clot adheres to wound sites, forming a barrier to fluid leaks and generates adhesion between tissues, while providing hemostatic and healing properties to the treated site.

Presently marketed, applicant's CryoSeal™ system is a device which harvests cryoprecipitated, concentrated clotting and adhesive proteins, including fibrinogen and Factor XIII from a donor's plasma in approximately one hour. The one hour cryoprecipitation harvesting, provided by the CryoSeal™ system, compared to the 1 to 2 day cryoprecipitation process routinely practiced in Blood Banks, means that CryoSeal™ harvesting of clotting and adhesive proteins can occur right in the perioperative theater with the patient close by, thereby avoiding the need to initiate the process days in advance.

These CryoSeal™ harvested clotting and adhesive proteins, when combined with bovine or human thrombin, forms a biological glue useful for surgical hemostasis and tissue adhesion. Commercially available thrombin, however, is generally sourced from bovine or human plasma pools, so the patient would still be at risk of negative immune reactions or contamination by infectious blood born viruses and, possibly Crutzfeld-Jacobs Disease (CJD) or new variants of CJD (NVCJD). An advantage of the CryoSeal™ cryoprecipitation invention is that the harvested clotting and adhesive proteins sourced from the patient's own blood eliminates the risk of contamination by infectious blood-borne disease when these clotting and adhesive proteins are topically applied to the patient's surgical wound sites.

It has long been understood, however, that the safest condition for a surgical patient would result from a two component biological sealant preparation in which the thrombin component would be harvested from the same donor in which the clotting and adhesive protein component was harvested - forming a fully autologous biological sealant or glue.

For instance, Cederholm-Williams PCT Patent (WO94/00566 - 6 January 1994) clearly describes an improved fibrin glue in which the thrombin component whose preparation method, - adjusting the ionic strength of the blood and pH of the plasma to cause precipitation of a thrombin component for later resolubilization - was described therein, would be combined with a fibrinogen component also sourced from the plasma of the same donor. These steps are so time consuming they become impractical for use in the perioperative theater where processing times should be less than one hour.

Three years later, in 1997, Hirsh, et al. (U.S. Patent No. 5,643,192) follows the lead of Cederholm-Williams by also teaching a method of preparing fibrin glue in which both the fibrinogen and thrombin components of a fibrin glue are sourced from the same donor's plasma. The Hirsh patent describes a method of preparing thrombin in which the fibrinogen in the plasma is first precipitated to prepare a supernatant and then clotting the residual fibrinogen in the supernatant which is different than the method taught by Cederholm-Williams, but does not result in a commercially useful thrombin in that (see figure 1 of Hirsh, et al.) the thrombin provides clotting speeds of five seconds or less for only 4 minutes, and less than 10 seconds for only 47 minutes.

These clotting speeds are unsuitable to the needs of surgeons who could not plan their entire surgeries around the limitations of the Hirsh, et al. fibrin glue.

Surgeons predominately require a fast acting clotting time (< 5 seconds) for hemostasis and tissue sealing or adhesion. Slow clotting biological glues (> 5 seconds) will often be transported away from the wound site by oozing and bleeding before they can perform their function. A surgeon utilizing the Hirsh fibrin glue would be required to arrange his surgery so that the hemostasis and tissue sealing intended for treatment with the Hirsh fibrin glue would occur within the 4 minute window where the clotting time was less than 5 seconds, making the Hirsh invention totally impractical for most surgeries which predominantly require rapid hemostasis and tissue adhesion throughout the surgery, the time span of which could extend to six hours.

The following prior art reflects the state of the art of which applicant is aware and is included herewith to discharge applicant's acknowledged duty to disclose relevant prior art. It is stipulated, however, that none of these references teach singly nor render obvious when considered in any conceivable combination the nexus of the instant invention as disclosed in greater detail hereinafter and as particularly claimed.

U.S. PATENT DOCUMENTS

<u>PATENT NO.</u>	<u>ISSUE DATE</u>	<u>INVENTOR</u>
5,648,265	July 15, 1997	Epstein
5,510,102	April 4, 1996	Cochrum
5,585,007	December 17, 1996	Antanavich, et al.
5,605,887	February 25, 1997	Pines, et al.
5,614,204	March 25, 1997	Cochrum
5,631,019	May 20, 1997	Marx
5,643,192	July 1, 1997	Hirsh, et al.

FOREIGN PATENT DOCUMENTS

<u>PATENT NO.</u>	<u>ISSUE DATE</u>	<u>INVENTOR</u>
-------------------	-------------------	-----------------

WO 94/00566
EU 0 592 242 A1

January 6, 1994
April 13, 1994

Cederholm-Williams, et al.
E.R. Squibb & Sons

The other prior art listed above, not all of which are specifically discussed catalog the prior art of which the applicant is aware. These undiscussed references diverge even more starkly from the instant invention specifically distinguished below.

0970927-14000

SUMMARY OF THE INVENTION

The instant invention addresses the long felt need for a simple, practical, fast method of preparing stable human thrombin from a donor's blood, which will provide fast clots (< 5 seconds) throughout a lengthy surgery (e.g. six hours) to combine with the clotting and adhesive proteins harvested and concentrated from the same unit of blood to form a biological sealant with no patient exposure to microbial or possible CJD or NVCJD contaminations. Previous works in the field (Hirsch, et al.) exemplified a thrombin with minimal stability in that the thrombin achieved rapid clotting of fibrinogen (i.e., less than 5 seconds) during only a very narrow four to five minute time period, totally impractical for the broad range of surgeries.

The present invention provides a stable thrombin enzyme which can cause precise, repeatable fast or slow polymerization of clotting and adhesive proteins over a duration of up to six hours - throughout even a long surgery. Further, the use of clotting and adhesive proteins and thrombin all sourced from a single donor will eliminate various disease risks posed from the use of commercial fibrin glues where the fibrinogen is sourced from plasma pooled from thousands of donors and the thrombin is either sourced from a similar pool of human plasma or of bovine origin. The speed and simplicity of the production of stable thrombin by use of this invention allows it to be prepared just prior to or during operative procedures and it will provide fast clotting throughout even the longest surgeries. The thrombin produced by this invention can be diluted in saline to provide precise, slower clotting times thereby allowing any preferred time from less than four seconds to longer than 2 minutes.

The procedure of the invention is comprised of three steps, the first two of which should occur at the same time:

1. Preparing a fraction enriched in prothrombin by use of Ethanol to substantially enhance the concentration of prothrombin and at the same time remove or denature naturally occurring ingredients within plasma, such as Thrombinodulin and Antithrombin III which can bind to, block, interfere with or inhibit prothrombin or its subsequent activation to long-term functional thrombin.

2. Adding calcium ions to the enriched prothrombin solution and briefly agitating the solution to convert the pro-thrombin to stable, long term thrombin.

3. Expressing the thrombin solution through a filter to remove particulate matter which would prevent spraying the thrombin through a small orifice or expressing the thrombin through a thin tube onto a wound site.

OBJECTS OF THE INVENTION

Accordingly, it is a primary object of the present invention to provide a new and novel apparatus and method to derive fast acting, stable autologous thrombin from the donor's plasma.

It is a further object of the present invention to provide thrombin as characterized above which has a shelf life longer than most associated surgical procedures.

It is a further object of the present invention to provide thrombin as characterized above in which the clotting time can be predictably lengthened at will through dilution with saline.

It is a further object of the present invention to provide thrombin as characterized above which has simple preparatory procedures.

It is a further object of the present invention to provide a method for producing thrombin as characterized above which has a process time of less than thirty minutes.

It is a further object of the present invention to provide thrombin which can be sprayed through small orifices or expressed through thin tubes.

Viewed from a first vantage point it is the object of the present invention to provide a novel and practical method for producing stable human thrombin from a prothrombin fraction which has been substantially enriched by ethanol fractionation to increase the prothrombin concentration and at the same time remove contaminating proteins. The addition of calcium chloride to the enriched prothrombin converts prothrombin to thrombin. From the same sole donor plasma, clotting and adhesive proteins are simultaneously obtained by other means to comprise the second component necessary for the autologous biological sealant.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a perspective view of an apparatus for sequestering prothrombin from plasma, processing the prothrombin into thrombin and taking the plasma not relegated towards the prothrombin and extracting clotting and adhesive proteins therefrom.

Figure 2 is a sectional view of the thrombin processing unit where plasma is being admitted into a mixing syringe.

Figure 3 is a view similar to figure 2 in which the processing reagents are directed to the mixing syringe for processing the plasma into prothrombin and then thrombin.

Figure 4 is a view similar to figures 2 and 3 where the thrombin is directed to a dispensing syringe after have first been filtered for particulate matter which could interfere with the thrombin being sprayed through a small orifice or expressed through a thin tube.

Figure 5 is a chart illustrating the effect of various ETOH concentrations in the final volume on the life span of fast clotting thrombin when the CaCl_2 is held constant at 0.023 μm .

Figure 6 is a chart illustrating the effect of various CaCl_2 concentrations in the final volume on the life span of fast clotting thrombin when the ETOH concentration is held constant at 13.6% .

Figure 7 is a chart illustrating that the processing of the thrombin should occur in a glass syringe for a fast clotting preparation.

Figure 8 is a chart describing the contaminating proteins removed from the enriched thrombin fraction after mixture with ETOH, (13.6% in final volume) and CaCl_2 (.023 μm in final volume) and filtered for particulate matter.

Figure 9 is a view showing the life span of optimized thrombin preparation for fast clotting.

Figure 10 is a view showing the life span of optimized thrombin preparation diluted at 1:15 with sterile saline for slow clotting.

Figure 11 is a chart illustrating the conversion/activation period required for the enrichment of a prothrombin fraction and its conversion to stable thrombin by mixture with a precise solution of ETOH and CaCl_2 .

Figure 12 is a chart illustrating thrombin (Bovine) concentrations (activity) as it relates to speed of clotting.

DESCRIPTION OF PREFERRED EMBODIMENTS

Referring to the drawings, wherein like elements denote like parts throughout, reference numeral 10 is directed to the processing set according to the present invention and shown in figure 1.

In its essence, the processing set 10 includes a fluid receiving system 20 which communicates with both a thrombin processing unit 40 and a clotting and adhesive proteins processing unit 60.

More particularly, the fluid receiving system 20 includes an inlet 2 communicating with tubing 4 through which plasma will enter the processing units 40, 60. The conduit 4 has a stop valve 6 which can occlude the tubing 4 preventing fluids through passage. The tubing 4 communicates through a T fitting 8 to divide plasma into two branches, a first branch 12 which leads to the thrombin processing unit 40 and a second branch 14 leading to the clotting and adhesive proteins processing unit 60.

Since it is preferred that the blood product admitted to the inlet 2 be plasma, the whole blood is first processed either by filtering, centrifugation, or another means of settling to remove the heavier red blood cells from the blood products, leaving plasma therebeyond for use in the figure 1 device. The plasma required for the thrombin processing unit is preferably 8 ml. so that the final volume of concentrated thrombin matches a typical yield of cryoprecipitated clotting and adhesive proteins from the clotting and adhesive proteins processing unit 60. Referring to figure 2, a sealed bag 16 overlies the thrombin processing unit 40 to provide sterility until the thrombin storage syringe is introduced into a sterile surgical field. Prior to that, the thrombin processing unit is operated as shown in figure 2 within the sealed bag which is flexible and sized to preferably permit the

movement of the syringes' plungers from the exterior of the bag. Fluid from the first branch 12 passes beyond a coupling 18 and into a manifold 22. The manifold 22 is equipped with a valve 24 that initially is directed to a mixing syringe 26 preferably formed from glass and capable of receiving a volume as great as 15 ml. The mixing syringe 26 includes a plunger 28, which when moved in the direction of the arrow A, draws the plasma from the passageway 12 and into the interior of the mixing syringe 26.

Referring to figure 3, the valve 24 is reoriented so that access can be gained between the mixing syringe 26 and the reagents found in ampoules 32, 34, each of which are operatively connected to the manifold 22 via a Y coupling 36 shown in figure 1. Access to the interior of either ampoule 32 or 34 can be had by squeezing the ampoule to rupture a frangible diaphragm. Alternatively, the intake 38 which receives the ampoule can be provided with a hollow spike which penetrates the diaphragm. In either event, the contents of both of the ampoules 32, 34 are received in the mixing syringe 26 by further retraction of the plunger 28 along the arrow A shown in figure 3. A first ampoule 32 is preferably provided with 2 ml. of ethanol providing an ETOH concentration in the final volume of 13.6% and the second ampoule 34 is preferably provided with 1 ml. calcium chloride providing a concentration in the final volume of .023μm. Alternatively, these reagents contained within the two ampoules 32, 34 can be premixed into a single ampoule and dispensed simultaneously. In one form of the invention, it is possible to introduce the ethanol first, then agitate the mixing syringe 26 and then follow with the calcium chloride, but the introduction of both simultaneously to the plasma are optimally combined, followed by brief agitation.

Once the ethanol and calcium chloride have been introduced into the mixing syringe 26, the valve 24 is reoriented so that the mixing syringe 26 is isolated. The contents are briefly agitated and allowed to incubate for about 20 minutes. Prior to pushing the contents out of the mixing syringe 26, the valve 24 is reoriented as shown in figure 4 after which the plunger 28 is moved in the direction of the arrow B of figure 4. Because the valve 24 is now set to allow communication to the thrombin dispensing syringe 42, the contents within the mixing syringe 26 will be transferred from the mixing syringe 26 to the dispensing syringe 42. More specifically, the manifold 22 includes a recess within which a filter 44 is provided in the flow path between the mixing syringe 26 and the thrombin dispensing syringe 42. Particulate matter will be retained within the filter 44 prior to delivery of the thrombin to the dispensing syringe 42. Note that as fluid enters the dispensing syringe 42, the dispensing syringe plunger 46 moves in a direction opposite arrow B.

Referring back to figure 1, attention is now directed to the clotting and adhesive protein processing unit 60. All of the plasma not diverted to the thrombin processing unit 40 is admitted to an interior chamber 62 of the clotting and adhesive protein processing unit 60. The clotting and adhesive protein processing unit 60 is manipulated by heat exchange and rotation so that all clotting and adhesive proteins extracted from the plasma will sediment at a nose 64 of the bag 62 for subsequent extraction by means of a clotting and adhesive protein dispensing syringe 66 contained in a sterile pouch 68. Once the thrombin has been loaded into the dispensing syringe 42, and the clotting and adhesive proteins have been loaded into the clotting and adhesive dispensing syringe 66, the two syringes can be decoupled from the processing set 10 and ganged together for spraying or line and dot

application. Mixing the thrombin with the clotting and adhesive proteins forms the biological glue.

Both dispensing syringes should be stored at or below 4°C prior to usage.

Turning to figure 5, a graph is shown which illustrates how ethanol concentrations alter the life span of fast clotting thrombin where the calcium chloride content is held constant at point .023 μ m. Note that at approximately 13.6% ethanol, its life span is shown to have been optimized and extend at least 240 minutes while its clotting time is substantially constant at under 5 seconds. The range between 8% and 18%, however, has utility.

Figure 6 varies the calcium chloride concentration in the thrombin while the ethanol is held constant at 13.6%. As shown, the thrombin life span where the calcium chloride concentration is at .023 μ m of 250 mM calcium chloride appears optimized and extends to 360 minutes while maintaining a clot time under 5 seconds. The range between .011 μ m of 125mM and .045 μ m of 500mM, however, has utility.

Figure 7 reflects the differences in processing the thrombin where the thrombin mixing syringe 26 is formed from glass versus plastic. As can be shown, the speed of clotting is held to close to 5 seconds or less with a life span of 60 to 240 minutes in glass.

Figure 8 reflects the effect of using ethanol at 13.6% and calcium chloride at .023 μ m to reduce proteins which alter the clot time of the thrombin as compared to the original plasma. As can be seen in this graph, the major interfering proteins are so efficiently removed, that the clotting time of the thrombin is not only enhanced, but held substantially stable and constant.

Figure 9 shows in greater detail than that which is shown in figures 5 and 6 regarding the measured clot time as a function of life span for the optimized thrombin preparation, having been treated by 13.6% ethanol and .023 μ m calcium chloride. As shown, the life span extends to 360 minutes and the clot time varies from 3 to 4 seconds.

Figure 10 shows the effect of saline solution of the thrombin preparation optimized as in figure 9 with an ethanol concentration of 13.6% and a calcium chloride concentration of .023 μ m as a function of life span. When the thrombin has been diluted 1 to 1.5 with saline, the clot time has been extended from just above 20 seconds to just less than 30 seconds, and has a life span of up to 150 minutes.

Referring to figure 11, there shown is the benefit in allowing the thrombin contained in the mixing syringe 26 to reside therein after agitation for almost 20 minutes in order to assure the effectiveness of the filtration step in removing particulate matter for subsequent utilization. The time span for conversation and activation allows enough particulate matter to be removed by the filter to optimize the use of the thrombin later in a narrow orificed dispenser, such as a sprayer or expressing through a thin tube.

Figure 12 provides a prior art comparison of the activity of thrombin sourced from Bovine blood plasma as it relates to the speed of clotting, showing that autologous thrombin derived from this invention provides a clotting speed equivalent to 100 iu/ml of Bovine thrombin.

Moreover, having thus described the invention, it should be apparent that numerous structural modifications and adaptations may be resorted to without departing from the scope and fair meaning of the instant invention as set forth hereinabove and as described hereinbelow by the claims.

CLAIMS

I Claim:

Claim 1 - A method for generating autologous thrombin from a patient, the steps including:

obtaining a blood product from the patient;
sequestering plasma from the product;
enriching the prothrombin in a plasma fraction;
converting the prothrombin to thrombin, and
filtering particulate from the thrombin.

Claim 2 - The method of claim 1 further including the step of altering the clotting time.

Claim 3 - The method of claim 2 including adding ethanol to enrich the prothrombin in a plasma fraction.

Claim 4 - The method of claim 3 wherein the converting step includes adding CaCl_2 .

Claim 5 - The method of claim 4 including centrifuging the blood product for obtaining plasma.

Claim 6 - The method of claim 2 wherein a predictable clotting time extension occurs through diluting the thrombin with saline.

Claim 7 - The method of claim 6 including filtering the plasma by weight size and protein binding.

Claim 8 - A method for producing autologous thrombin which is stable for more than 15 minutes, the steps including:

sequestering pro-thrombin from plasma and converting the pro-thrombin to thrombin.

Claim 9 - Autologous thrombin which provides fast clotting for more than 15 minutes.

Claim 10 - A composition for extracting thrombin from plasma consisting essentially of:

Plasma;

Ethanol (ETOH);

CaCl₂.

Claim 11 - The composition of claim 10 wherein ETOH is present at 13.6% and CaCl₂ is present at .023μm both by volume.

Claim 12 - A method for preparing thrombin comprising:

- obtaining plasma;
- adding ETOH and CaCl₂ to the plasma, forming a composition;
- agitating the composition;
- filtering the composition of particulate, thereby passing the thrombin through the filter.

Claim 13 - A manifold with an integrated filter for preparing thrombin from plasma, comprising:

a dock for a plasma syringe on the manifold;

a receptacle for a solution of CaCl₂ and ETOH and a dock on the manifold therefore;

a dock for a thrombin receiving syringe to receive the thrombin through the filter;

the integrated filter located on the manifold and just upstream the thrombin receiving syringe.

ABSTRACT

A sterile method for preparing stable thrombin component from a single donor's plasma in which the thrombin component is harvested simultaneously from the clotting and adhesive proteins component from the same donor plasma in less than one hour. The combined components provide an improved biological hemostatic agent and tissue sealant by virtue of its freedom from the risk of contaminating viruses or bacteria from allogenic human or bovine blood sources. The thrombin provides polymerization of the clotting and adhesive proteins in less than five seconds, and is sufficiently stable to provide that fast clotting over a six hour period. Further, the clotting times can be predictably lengthened by diluting the thrombin with saline.

UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Coelho, P., et al.
FOR:	Apparatus and Method of Preparation of Stable, Long Term Thrombin from Plasma And Thrombin Formed Thereby

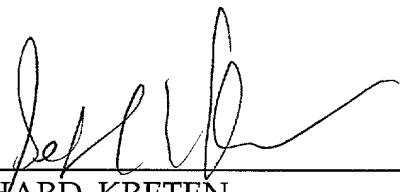
To: Commissioner of Patents and Trademarks
Washington, DC 20231

LETTER TO THE DRAFTSMAN

Subject to the Examiner's approval, kindly enter the enclosed twelve (12) sheets of formal drawing figures (comprised of figures 1 through 12) in place of the drawing figures as originally filed in the above-identified case.

Dated: November 9, 2000

Respectfully Submitted:



BERNHARD KRETEN
Applicant's Attorney
Telephone (916) 921-6181
Registration No.: 27,037

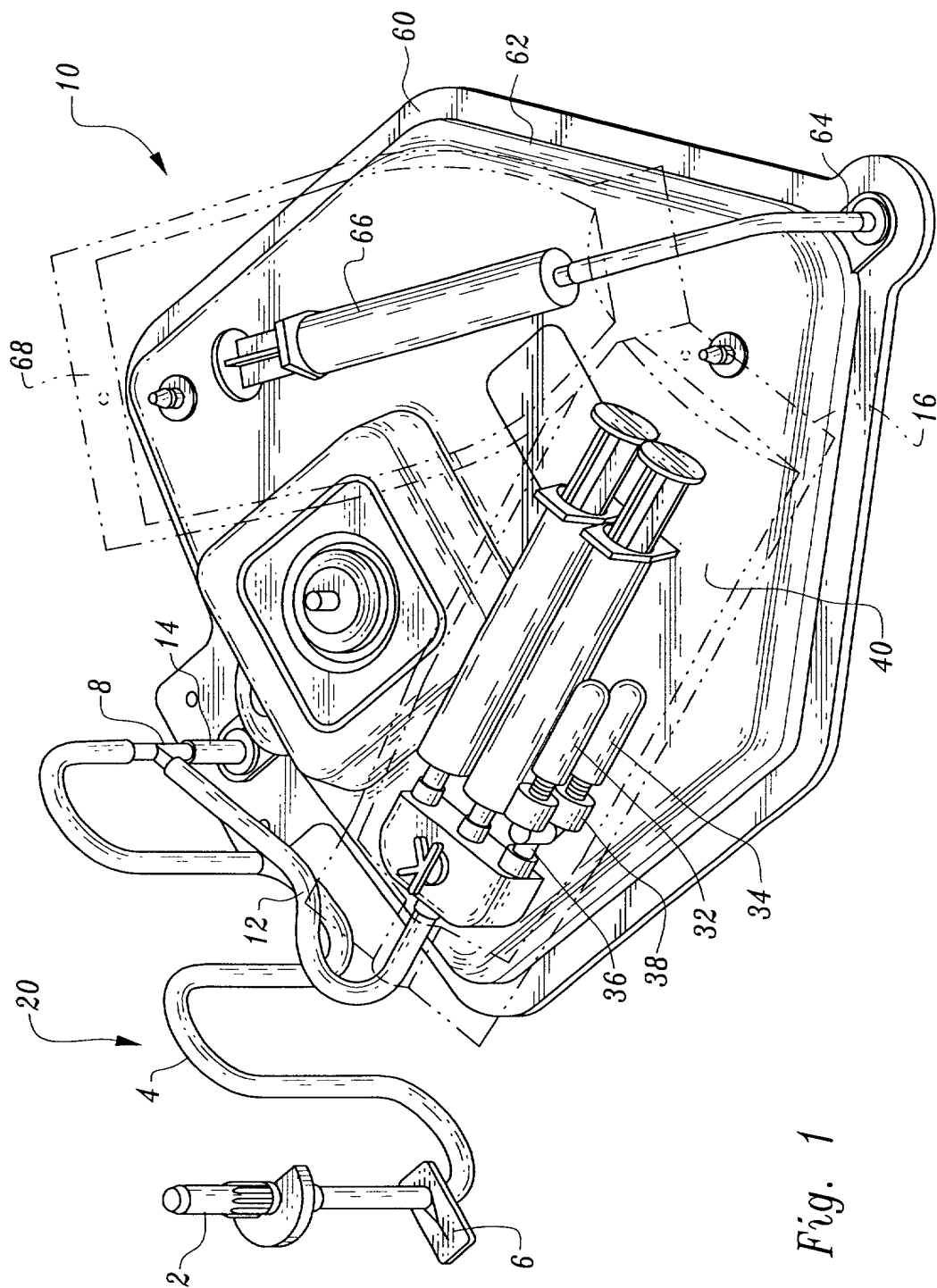


Fig. 1

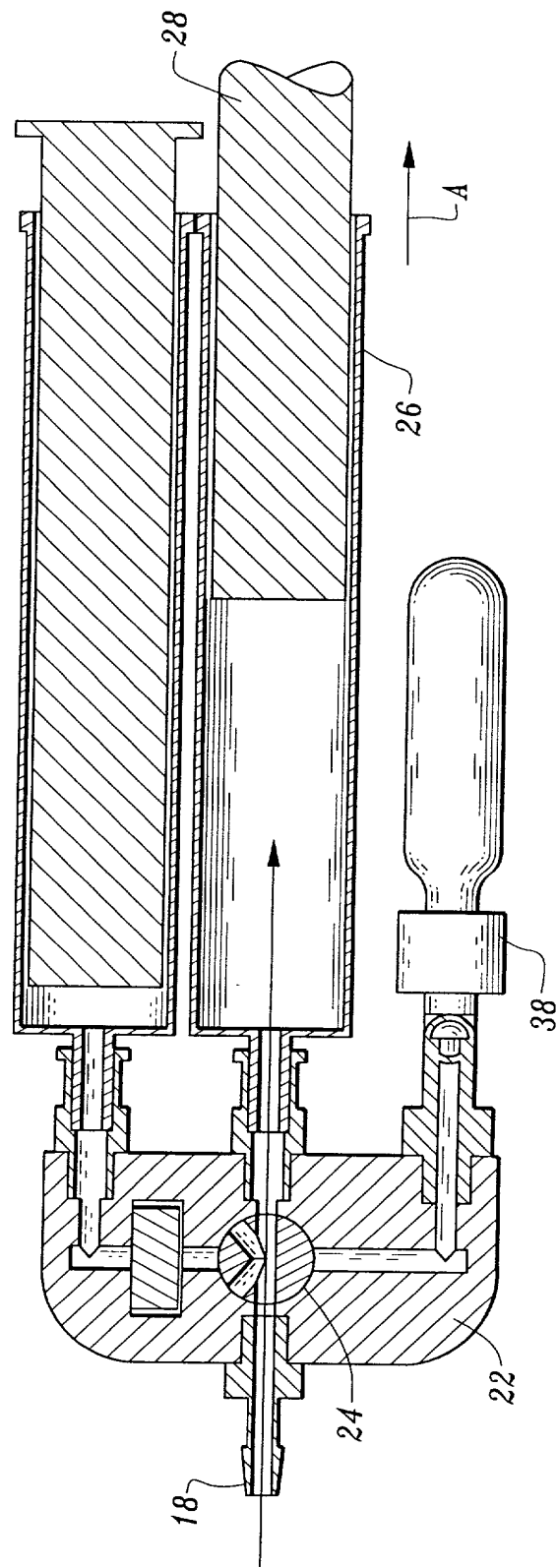


Fig. 2

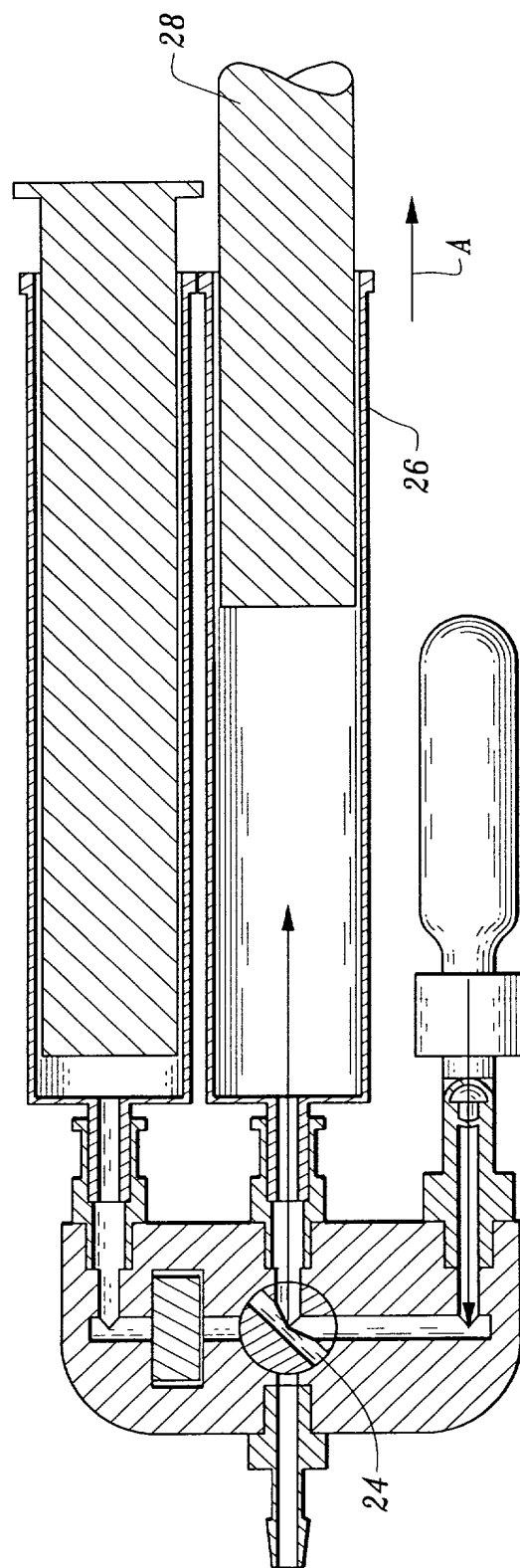


Fig. 3

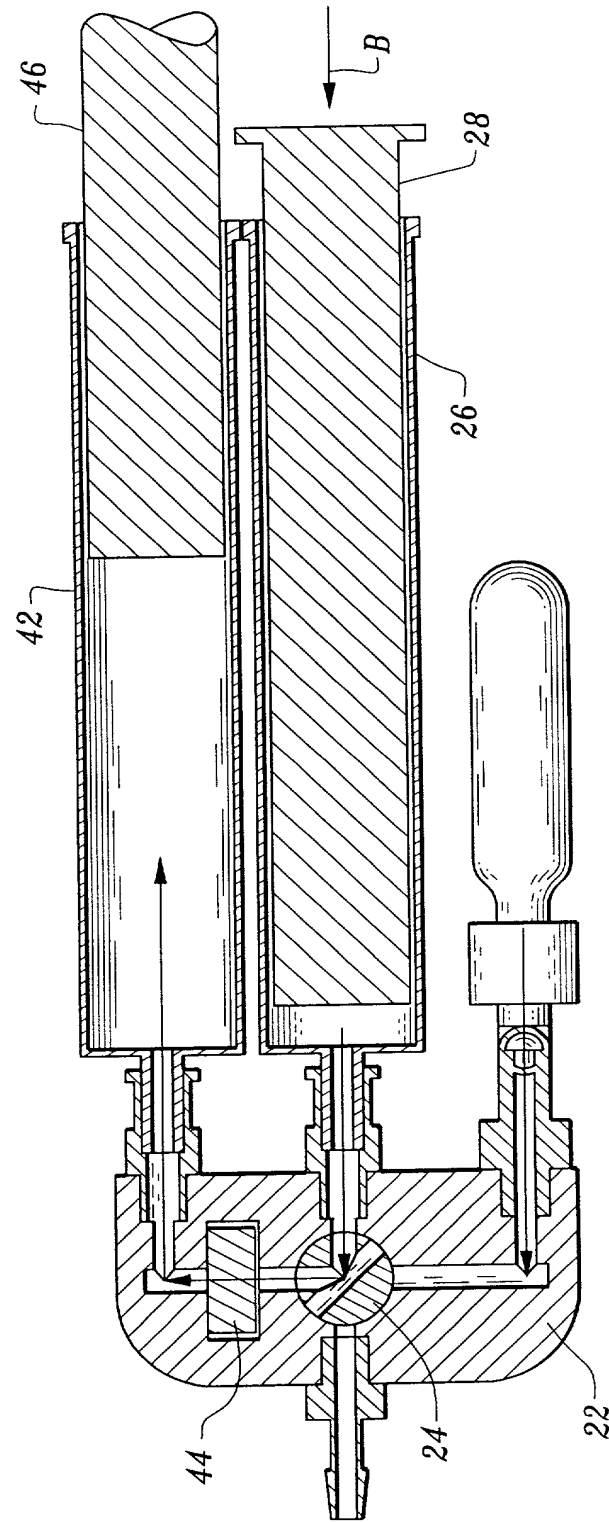


Fig. 4

LIFESPAN OF FAST CLOTTING THROMBIN FRACTIONATED AT DIFFERENT ETOH CONCENTRATIONS

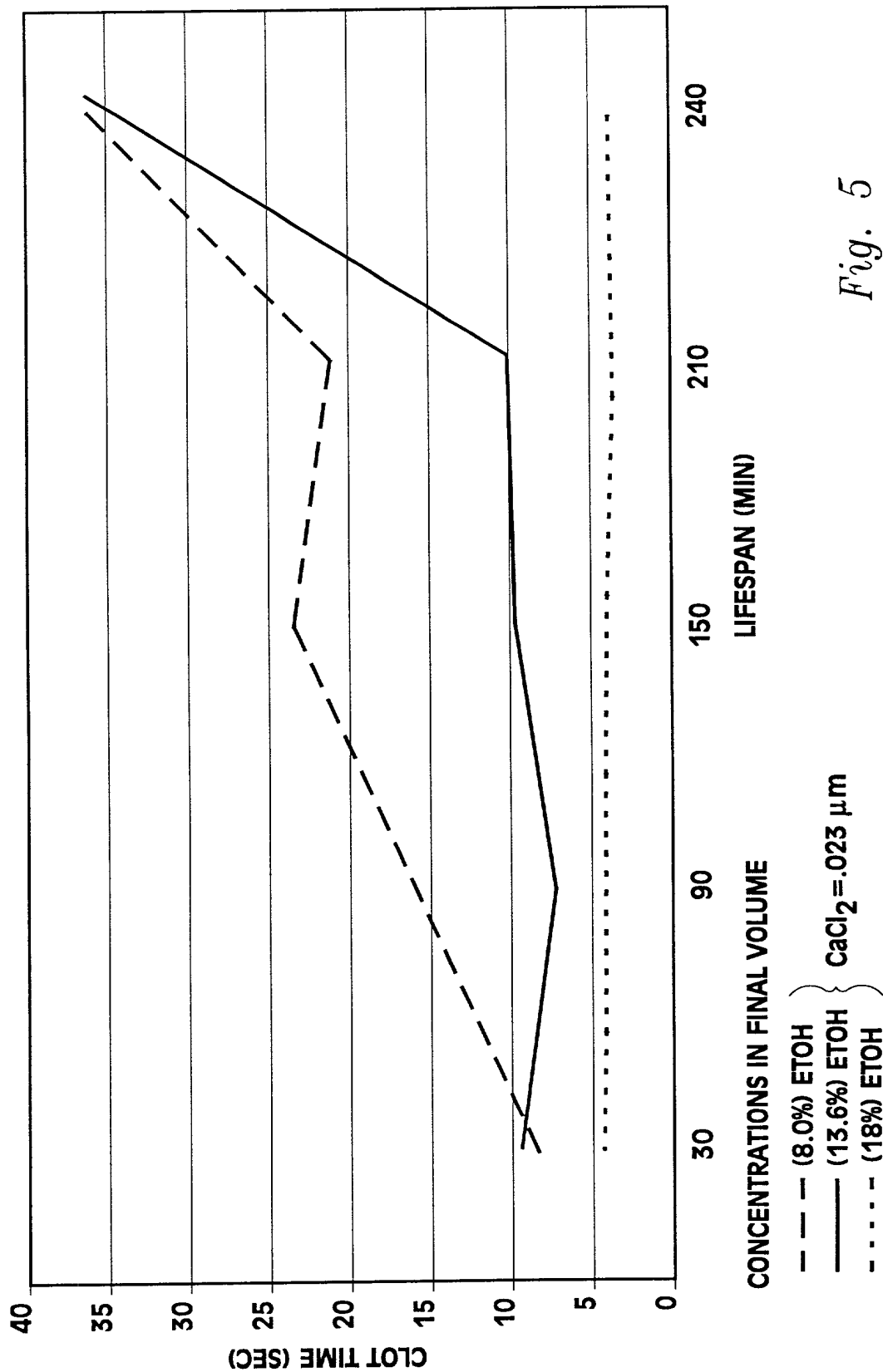
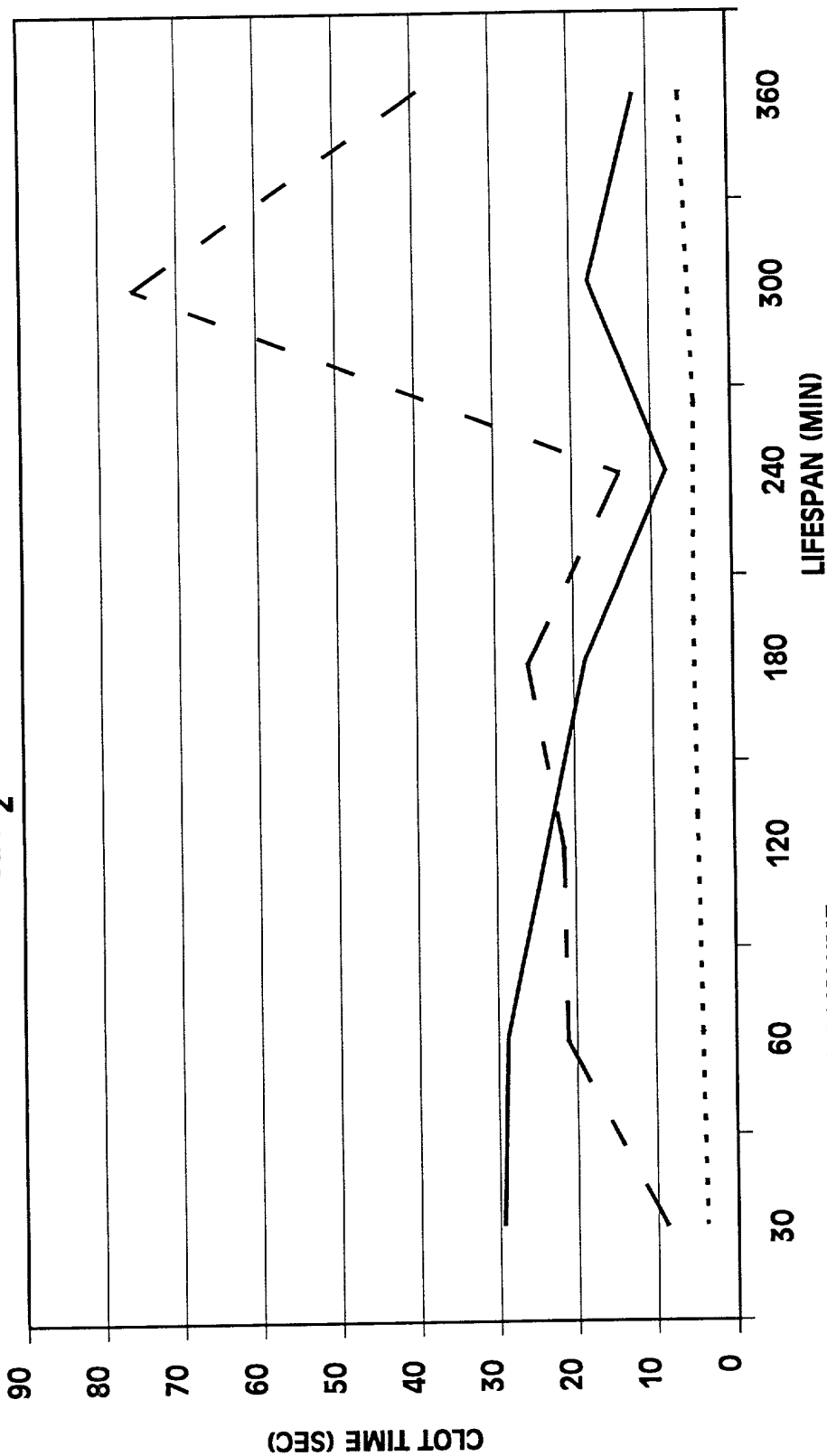


Fig. 5

LIFESPAN OF FAST CLOTTING THROMBIN AT DIFFERENT CaCl₂ CONCENTRATIONS

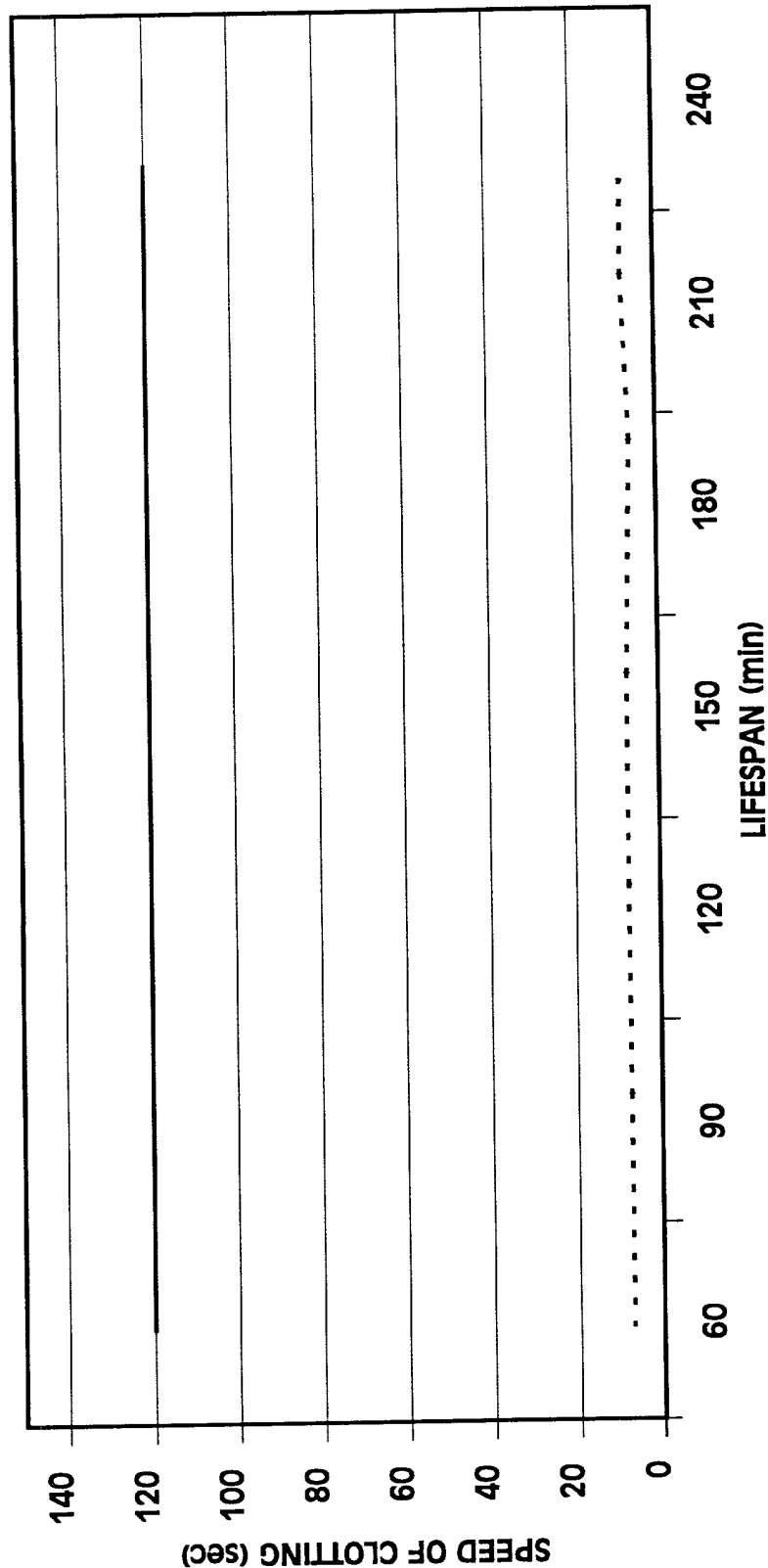


CONCENTRATIONS IN FINAL VOLUME

--- (0.11 μm) OF 125mM CaCl₂
 — (0.23 μm) OF 250mM CaCl₂
 - - - (0.45 μm) OF 500mM CaCl₂

Fig. 6

LIFESPAN AND CLOTTING SPEED OF THROMBIN PROCESSED IN CONTAINERS OF DIFFERENT MATERIALS



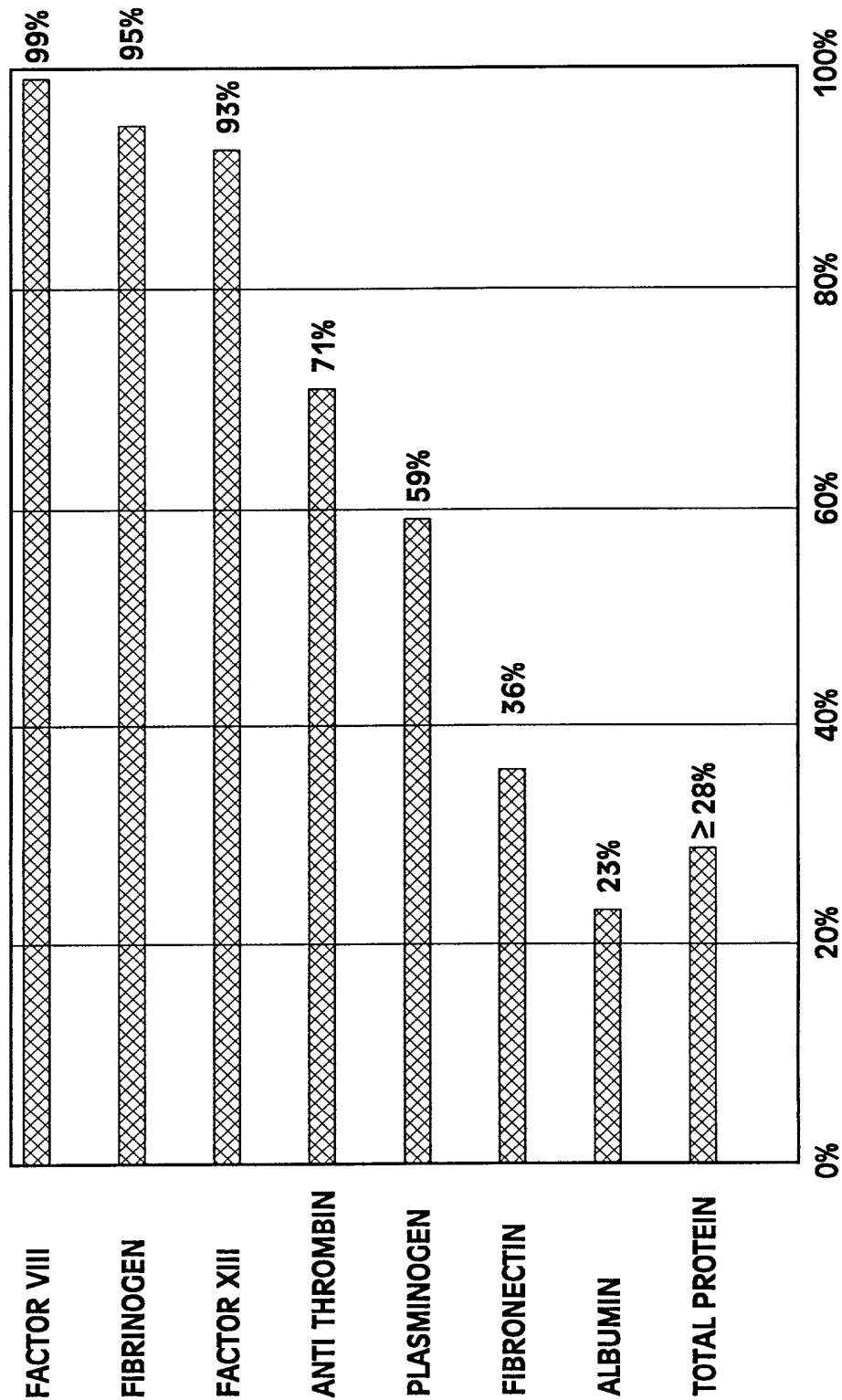
CONCENTRATIONS IN FINAL VOLUME

- ETOH 13.6%
- CaCl_2 .023 μm

— PLASTIC (neutral charge)
- - - - GLASS (negative charge)

Fig. 7

REDUCTION IN CONTAMINATING PROTEINS FROM OPTIMIZED ENRICHED THROMBIN FRACTION AS COMPARED TO THE ORIGINAL PLASMA

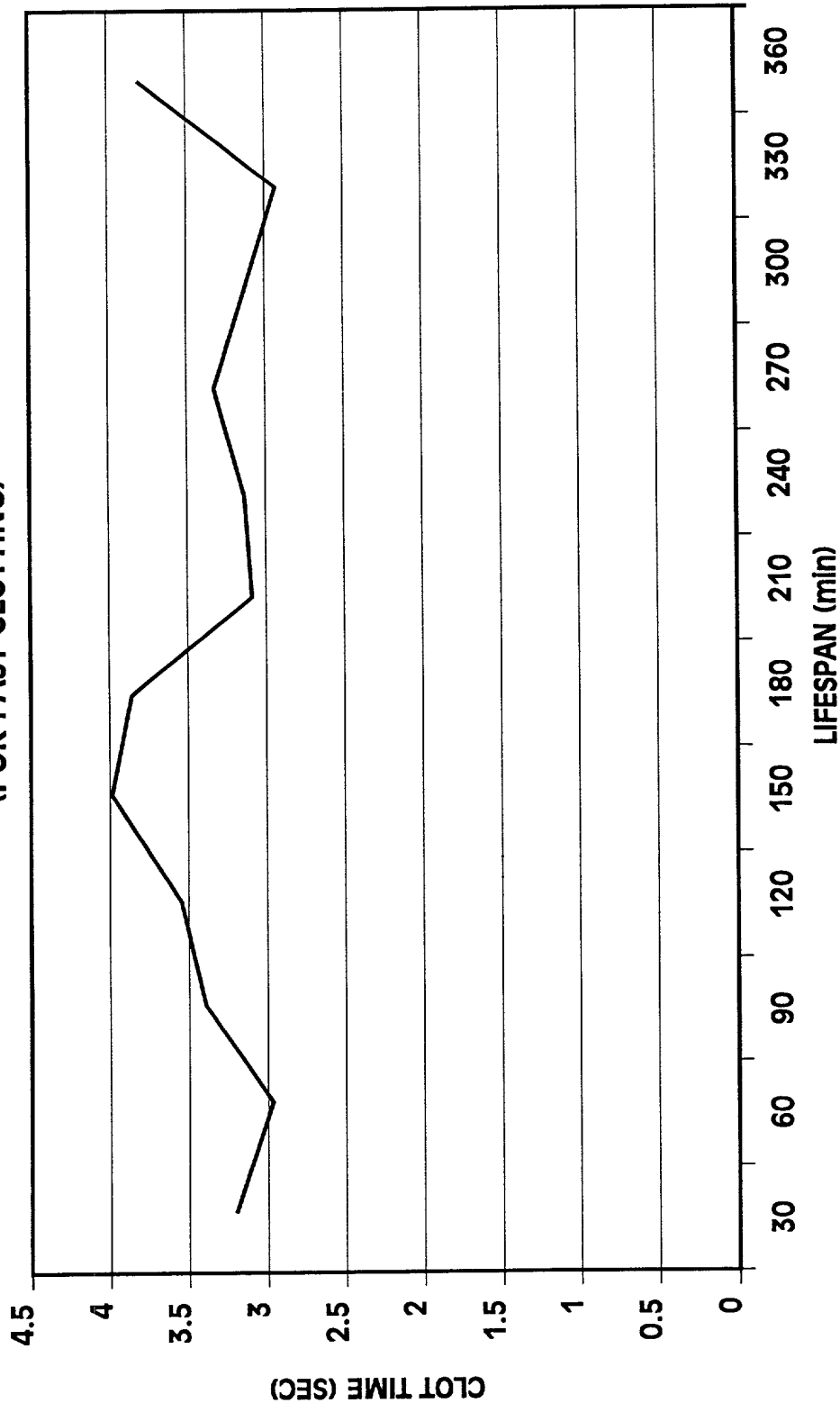


CONCENTRATIONS IN FINAL VOLUME

- ETOH 13.6%
- CaCl_2 .023 μm

Fig. 8

LIFESPAN OF OPTIMIZED THROMBIN PREPARATION (FOR FAST CLOTTING)

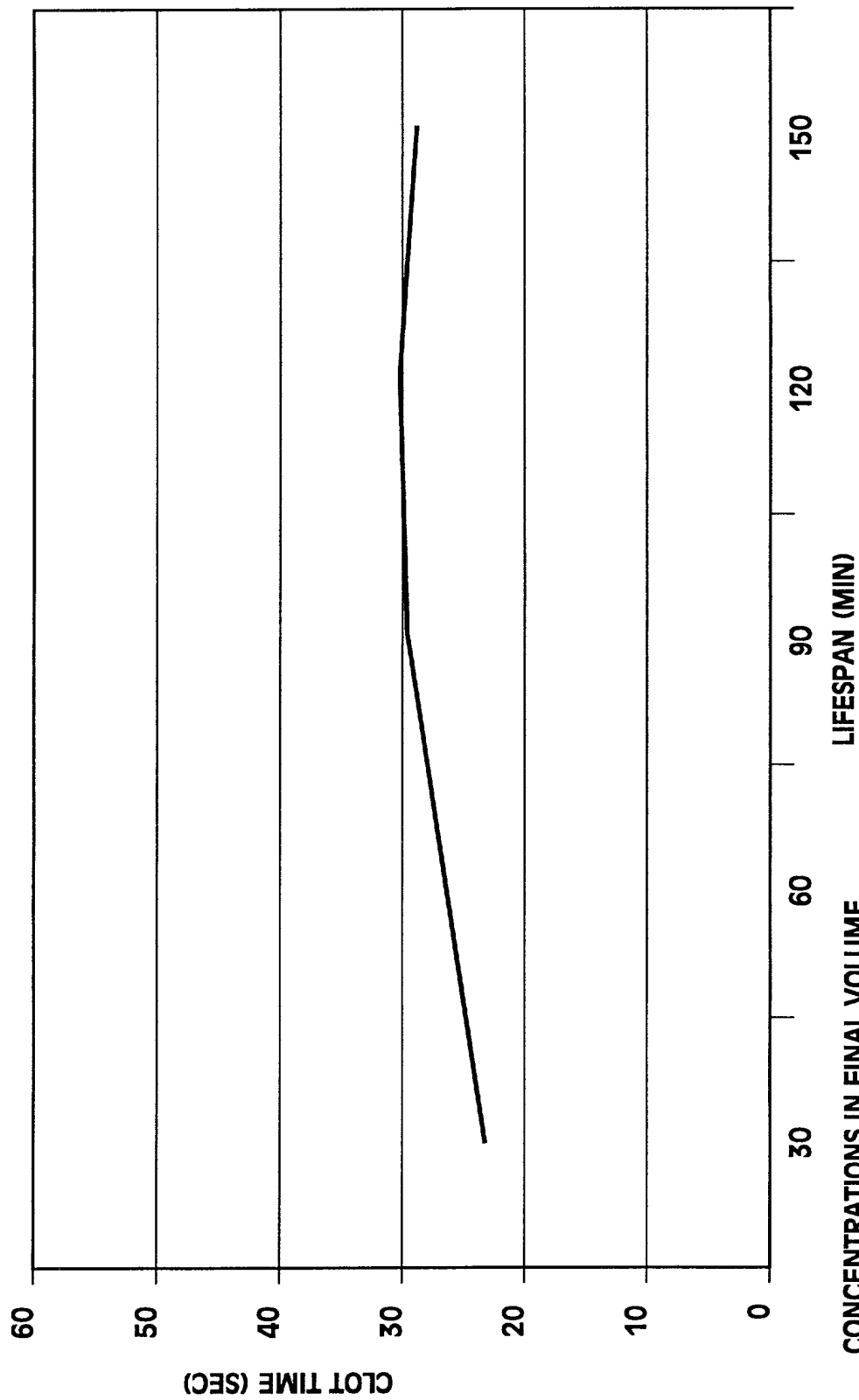


CONCENTRATIONS IN FINAL VOLUME

- ETOH 13.6%
- CaCl_2 .023 μm

Fig. 9

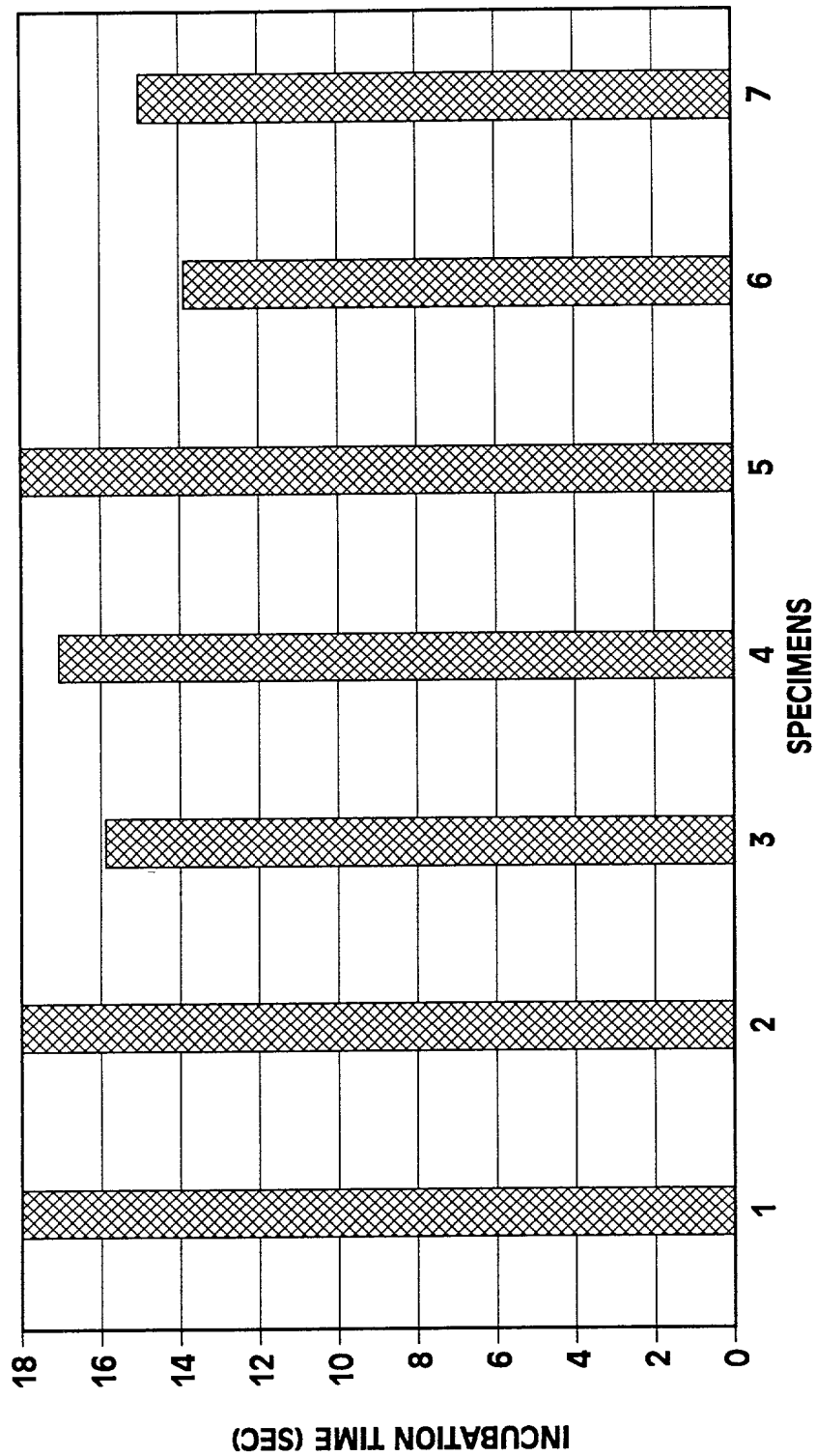
**LIFESPAN OF THROMBIN PREPARATION
(DILUTED 1:1.5 FOR SLOW CLOTTING)**



- ETOH 13.6%
- CaCl_2 .023 μm

Fig. 10

TYPICAL CONVERSION/ACTIVATION PERIODS TO CONVERT OPTIMIZED, ENRICHED
PROTHROMBIN FRACTION TO THROMBIN



CONCENTRATIONS IN FINAL VOLUME

- ETOH 13.6%
- CaCl_2 .023 μm

Fig. 11

**BOVINE THROMBIN
CONCENTRATION VS. CLOTTING TIME**

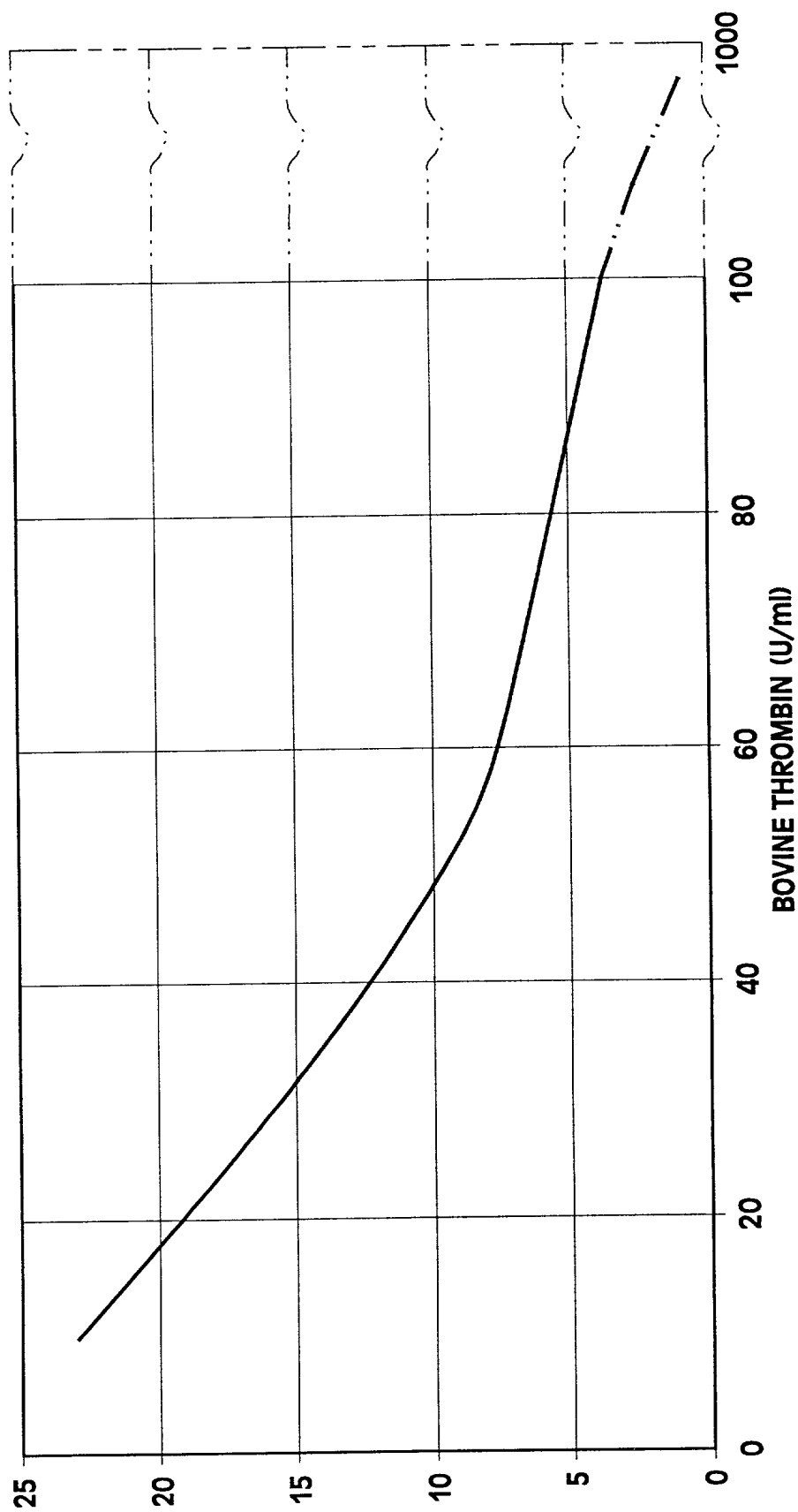


Fig. 12

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled Apparatus and Method of Preparation of Stable, Long Term Thrombin from Plasma and Thrombin Formed Thereby, the specification of which:

_____ is attached hereto.

XX was filed on August 5, 1998 as Application Serial No.: 09/129,988
and was amended on: _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37. (Code of Federal Regulations 1.56(a)).

I hereby claim foreign priority benefits under Title 35, U.S. Code 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)	Priority Claimed
	NO

(Number)	(Country)	(Day/Month/Year)
----------	-----------	------------------

I hereby claim the benefit under Title 35, U.S. Code 120 of any U.S. application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior U.S. application in the manner provided by the first paragraph of Title 35, U.S. Code 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, 1.56(a), which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status-patented, pending, abandoned)
--------------------------	---------------	---------------------------------------

I hereby appoint BERNHARD KRETEN, Reg. No. 27,037; VICTOR J. GALLO, Reg. No. 41,768; DENNIS A. DEBOO, Reg. No. 42,471; to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith.

Address all telephone calls to: (916) 921-6181

Address all correspondence to 77 Cadillac Drive, Suite 245, Sacramento, California 95825

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of Inventor: Philip Henry Coelho Citizenship: United States

Inventor's Signature: *Philip H. Coelho* Date: 10/6/98

Residence: 121 Giotto Way, El Dorado Hills, California 95762

Post Office Address: 121 Giotto Way, El Dorado Hills, California 95762

Full Name of Inventor: Phil Kingsley Citizenship: United States

Inventor's Signature: *Phil Kingsley* Date: 10/6/98

Residence: 9560 Appalachian Drive, Sacramento, California 95827

Full Name of Inventor: James H. Godsey Citizenship: United States
Inventor's Signature: James H. Godsey Date: 10-6-98
Residence: 101 Summer Shade Court, Folsom, California 95630
Post Office Address: 101 Summer Shade Court, Folsom, California 95630

A	
1	100%
2	98%
3	96%
4	94%
5	92%
6	90%
7	88%
8	86%
9	84%
10	82%
11	80%
12	78%
13	76%
14	74%
15	72%
16	70%
17	68%
18	66%
19	64%
20	62%
21	60%
22	58%
23	56%
24	54%
25	52%
26	50%
27	48%
28	46%
29	44%
30	42%
31	40%
32	38%
33	36%
34	34%
35	32%
36	30%
37	28%
38	26%
39	24%
40	22%
41	20%
42	18%
43	16%
44	14%
45	12%
46	10%
47	8%
48	6%
49	4%
50	2%
51	0%
52	0%
53	0%
54	0%
55	0%
56	0%
57	0%
58	0%
59	0%
60	0%
61	0%
62	0%
63	0%
64	0%
65	0%
66	0%
67	0%
68	0%
69	0%
70	0%
71	0%
72	0%
73	0%
74	0%
75	0%
76	0%
77	0%
78	0%
79	0%
80	0%
81	0%
82	0%
83	0%
84	0%
85	0%
86	0%
87	0%
88	0%
89	0%
90	0%
91	0%
92	0%
93	0%
94	0%
95	0%
96	0%
97	0%
98	0%
99	0%
100	0%
B	
1	100%
2	98%
3	96%
4	94%
5	92%
6	90%
7	88%
8	86%
9	84%
10	82%
11	80%
12	78%
13	76%
14	74%
15	72%
16	70%
17	68%
18	66%
19	64%
20	62%
21	60%
22	58%
23	56%
24	54%
25	52%
26	50%
27	48%
28	46%
29	44%
30	42%
31	40%
32	38%
33	36%
34	34%
35	32%
36	30%
37	28%
38	26%
39	24%
40	22%
41	20%
42	18%
43	16%
44	14%
45	12%
46	10%
47	8%
48	6%
49	4%
50	2%
51	0%
52	0%
53	0%
54	0%
55	0%
56	0%
57	0%
58	0%
59	0%
60	0%
61	0%
62	0%
63	0%
64	0%
65	0%
66	0%
67	0%
68	0%
69	0%
70	0%
71	0%
72	0%
73	0%
74	0%
75	0%
76	0%
77	0%
78	0%
79	0%
80	0%
81	0%
82	0%
83	0%
84	0%
85	0%
86	0%
87	0%
88	0%
89	0%
90	0%
91	0%
92	0%
93	0%
94	0%
95	0%
96	0%
97	0%
98	0%
99	0%
100	0%

